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Antibody Recognition of a Conformational Epitope in a Peptide Antigen of the Tumor-Associated Variant of the Epidermal Growth Factor Receptor, EGFRvIII

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Beamline(s): X8C

Introduction: The epidermal growth factor receptor mediates cell proliferation and differentiation. Many abnormal cells have been shown to express mutant forms of this receptor. The most common of those mutants, EGFRvIII, is characterized by a 801-bp in-frame deletion, which removes N-terminal amino acid residues 6 through 273 in the extracellular domain and produces a novel glycine residue at the junction. Synthetic peptides corresponding to this tumor-specific extracellular sequence have been used to produce monoclonal and polyclonal antibodies specific for EGFRvIII. One antibody variable fragment (Fv) in particular, called MR1, shows high affinity for both the EGFRvIII synthetic peptide and mutant cell surface receptor and does not show any detectable binding to the wild-type receptor (Lorimer *et al.*, 1996). This antibody is important in that it has the potential to be used as therapeutic agent in cancer patients for targeting of bacterial toxins. Understanding the mechanisms of recognition of this antibody is thus an important goal.

Methods and Materials: We crystallized MR1 in complex with a 12-mer synthetic EGFRvIII peptide. Crystals of the Fv-peptide complex are shown in Figure 1. Data sets were collected at room temperature (RT) at Queen's University, Kingston, Canada and at liquid nitrogen temperature (LT) at the National Synchrotron Light Source of BNL. The use of beamline X8C allowed us to collect diffraction data to high resolution and thus refine the model obtained with the RT data set.

Results and Conclusions: Molecular replacement was used and the 1.8 A resolution data set was refined to an R-factor of 0.164. Interestingly, the structure shows that the most distinctive portion of the peptide antigen, the novel fusion glycine, makes no contact to the Fv and does not contribute to the epitope. The specificity of MR1 lies in the ability of this glycine residue to assume a restricted conformation, and demonstrates that a peptide antigen can be used to generate a conformational epitope.

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References: I. A. Lorimer, A. Keppler-Hafkemeyer, R. A. Beers, C. N. Peegram, D. D. Bigner and I. Pastan, "Recombinant Immunotoxins Specific for a Mutant Epidermal Growth Factor Receptor: Targeting With a Single-chain Antibody Variable Domain Isolated by Phage Display," Proc. Natl. Acad. Sci. USA, **93**, *14815-14820*, 1996.

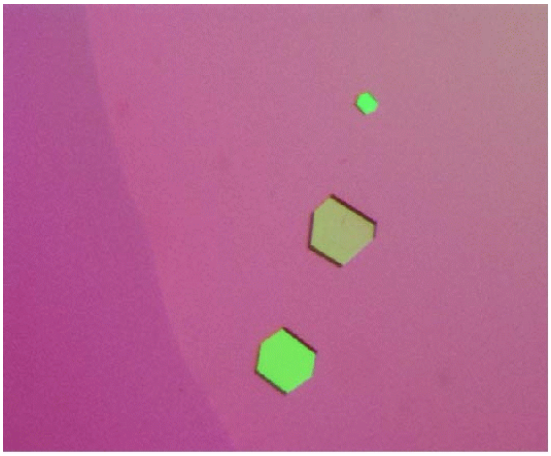

Figure 1. Crystals of the MR1dsFv-EGFRvIII peptide complex.